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Fine Structure of Blue-green Algae, *Microcystis aeruginosa* Kützing

Min Kyu Choi, Baik Ho Kim, Yeun Ja Mun, Yeun Tai Chung
Jong Bin Lee* and In Sun Wui*
Institute for Environmental Science, Wonkwang University, Iksan 570-749, Korea
*Department of Biology, Chonnam National University, Kwangju, 500-757, Korea

藍藻 *Microcystis aeruginosa* Kützing의 微細構造에 關한 硏究

崔玟圭 · 金白虎 · 文蓮子 · 鄭然泰 · 李鍾彬* · 魏仁善*
圓光大學校 環境科學硏究所, *全南大學校 生物學科
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요 약

주암호의 유입하천중 이화학적 조건이 서로 다른 보성천과 동복천에서 채집된 남조 *Microcystis aeruginosa* Kützing의 미세구조에 대한 형태학적 관찰을 시행하여 다음과 같은 결과를 얻었다. 수온과 pH를 제외한 다른 환경요인들은 두지점간에 큰 차이를 보였다. *M. aeruginosa*의 광학 및 전자현미경적 관찰 결과, 세포는 부정형 군체를 형성하며, 투명한 점액질로 싸여 있었다. 세포는 난형 또는 구형에 가깝고, 크기는 2.61~5.40 μm의 범위로서, 평균 3.54±0.19 μm였다. 세포질내에는 많은 다양한 구조물을 가지고 있으며, polyhedral bodies (carboxysomes), polyphosphate, cyanophycin granules 외에 photosynthetic lamellae와 gas vacuoles 등이 비교적 뚜렷하였다. 두 채집장소간의 형태학적 차이는 보이지 않았으나, 영양염류 수준이 높은 표본에서 polyphosphate와 cyanophycin granules이 숫적 증가를 보였다. 세포분열은 이분법으로 초기에 정중앙부의 좌우로부터 합입이 일어났으며, 주로 세포벽의 제1층과 제2층이 관여하였다. 세포벽의 외부층은 크게 두가지 형태의 filaments 층이 잘 발달되어 있었다.

Key words : Fine structure, *Microcystis aeruginosa*. Kütz. Blue-green algae, Lake Chuam

INTRODUCTION

Blue-green algae has a large number of different granules and inclusions, or storage bodies, of various structures and shapes, although their distribution is, in respect to that of bacteria, only partly Gram-negative (Wolks, 1973; Healy, 1982; Golecki and Heinrich, 1990). The chemical nature of some of these inclusion bodies has been identified with light microscopic staining methods or by chemical analyses (Hascoet *et al.*, 1985; Streichan *et al.*, 1990). The characteristics

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of these bodies under light and electron microscopy has been well known for the many kinds of blue-green algae used by most researchers doing culture experiments. Colonial *Microcystis aeruginosa* has been associated with numerous livestock deaths and implicated epidemiologically in human illness. They are known as hepato- and neurotoxins, and are more toxic in tests done on domestic animals than any other algal group (Aune and Berg, 1986; Galey *et al.*, 1987; Utkilen and Gjolme, 1992). These studies have focused more on the toxicity to experimental cells and tissues due to algal products, such microcystins (Wicks and Thiel, 1990), than on the morphological changes rendered by treatment with foreign materials. While there are a number of reviews on the blue-green algae (Wolk, 1973; Carr and Whitton, 1982), reports on the fine structures of *M. aeruginosa* were very few. Even so, the ultrastructural characteristics of *M. aeruginosa* have been examined mainly under culture conditions.

The purpose of this work was to examine the fine structures of *M. aeruginosa* taken from different natural habitats and to find a useful tool for classifying blue-green algae by genus.

MATERIALS AND METHODS

Lake Chuam (35°20′ ~34°43′N, 127°17′ ~127°05′E) is an artificial multipurpose, and dendrite type lake with many tributaries, surrounded by the provinces of Whasungun, Sungjugun and Bosunggun, located in the southwestern Korean Peninsula.

Sample collection was carried out at the mouths of Tongbok and Bosung streams at Lake Chuam on 13 September, 1994, which showed the highest counts of *Microcystis aeruginosa*. To collect *M. aeruginosa* colonies, a plankton net was used (Müller gauze No. 25, 50 ml).

For light microscopy, all samples were fixed with formalin acetic acid (FAA) solution or 5~7% neutral formalin solution. Before analysis, all samples were dispersed by ultrasonic disintegration (20 kHz, 60 sec). To count the total number of cells, a Sedgwick-Rafter Chamber was used under the light microscope with a camera system (Leitz, ×1,000).

For electron microscopy, colonial *M. aeruginosa* were collected, and 10 mL were fixed with 1% glutaraldehyde overnight. The samples were post-fixed with 1% buffered osmium tetroxide for 1.5 hrs. 0.1 M phosphate buffer with pH 7.0 was used for both fixatives. The fixed colonies were dehydrated in ascending grades of ethanol and embedded in epoxy resin. Thin sections were cut with a glass knife on an LKB ultamicrotome. The sections were stained with uranyl acetate, saturated in a solution of 50% ethanol followed by lead citrate, and examined with a Hitachi-600 electron microscope.

RESULTS AND DISCUSSION

Microcystis aeruginosa averaged 1.2×10^5 cells/ml accounting for 87.5% of total organism, and the number in the Tongbok branch was nearly twice that of Bosung. Thirty one phytoplankton assemblages were classified, among them: *Melosira granulata* and *Synedra ulna* in diatoms; *Scenedesmus quadricauda*, *Coelastrum microporum*, *Eudorina elegans* and *Pediastrum duplex* var. *reticulatum* in green alga; and *Peridinium willei* and *Ceratium hirundinella* dominated in dinoflagellates, but their numbers were below 1×10^3 cells/ml.

Light microscopy was used mainly for counting *M. aeruginosa* cells, distinguishing cell aggregates from mixed colonies, and for photographing

the cells. Natural *M. aeruginosa* colonies obtained from Lake Chuam, formed irregularly shaped non-directional arrays with distinct hyaline mucilage with individual cells either ovoid or spherical 3~5 μm in diameter. Many whitish granules or organelles, possibly gas vacuoles, were visible mainly on the outside of the cells, and sometimes, both the nucleoplasm and cytoplasm were divided into several parts by them (Fig. 1).

Under the electron microscope, *M. aeruginosa* colonies consisted of many kinds of cells, young and old, in various sizes and stages of division. Cells ranged from 2.61 to 5.40 μm in diameter, averaged 3.54±0.19 μm (n=311, $p<0.05$), and were either ovoid or spherical in shape (Fig. 2). In the cytoplasm, there were a number of inclusions of various sizes, shapes and appearances. Among inclusions, polyhedral bodies or carboxysomes, large or small structured and dense granules, photosynthetic lamellae or thylakoids, and gas vacuoles were prominent and easy to recognize (Figs. 3~9). Incidentally, there were large structured granules adhering to the cell surface (Fig. 2 and 3). We could not ascertain whether they were uptake of electron dense materials from extracellular medium, discharge from the cytoplasm, or phosphate sewage bacteria (Golecki and Heinrich, 1990). There were many granules diverse in shape and electron density in the cytoplasm of *M. aeruginosa*, which are known to be widely distributed in a number of blue-green algae (Figs. 6~9). These granules were categorized as follows; cyanophycin granules or structured granules, ploy phosphate granules, polyhedral bodies, glycogen granules, and ribosomes. Among them, the polyhedral bodies (PB) we observed (Fig. 9), often called crystalline bodies or carboxysomes, are known as vital for cell activities and have many functions (Mehta and Hawby, 1979). In ultrathin section, they varied in size and in the density of their electron fields, were normally seen in the center of the cell or near nuclear materials, and were hexagonal in shape.

There were no limiting or membranous structures around the PB's, but sometimes a definable interface covered them. The number and length of the PB's varied from 1 to 8, and 120~750 nm, respectively (Table 1, Fig. 4, 6 and 9). These bodies were a ubiquitous characteristic of the blue-green algae, often not only showing angular or irregular shapes, but also having a new membrane formation around the PB in many species (Mehta and Hawby, 1979). It is clear that the PB's always present in *M. aeruginosa*, have a morphological diversity of length, shape and number. Although more detailed experiments are need, we conclude for the time being that the PB's are a useful characteristic for blue-green algal classification of the genus level.

Until now, the ultrastructure of the outer membranes or outside cell wall of *M. aeruginosa* has been less understood than any other group, such as the unicellular cyanobacterium *Anacystis nidulans* (Golecki, 1974) or most Gram-negative bacteria (Weise *et al.*, 1970). The extracellular covering of *M. aeruginosa* was divided into several layers; the cytoplasmic membrane or plasmalemma, the peptidoglycan layer, and the multila-

Table 1. Numerical characteristics of polyhedral bodies.

characters	Mehta and Hawby (1979)			present study
	young cells	old cells	with Simazine	
No.	1~6 (1-3)	2~4 (2)	?	1~8 (5)
diameter	66~267 nm	80~360 nm	94~426 nm	120~750 nm
strains	*A. nidulans*			*M. aeruginosa*

( ): numbers in common or average

yered structure of the cell wall (Fig. 7). There were numerous filaments or filamentous projections, consisting of two parts, with the proximal portion of each appearing thicker than the distal one. Also, the filaments were approximately 0.136 μm in length and 0.056 μm in diameter (Fig. 7), similar to those of cyanobacterium *A. nidulans* (Golecki, 1977). These filaments are morphologically similar to pili previously known to exist among algal and bacterial groups such as *Synechocystis* and *Anabaena*. As Table 2 shows, there are considerable differences in the microscopic morphometry of the genus blue-green algae. This indicates that the outer layer of the cell wall may also be a useful characteristic for blue-green algal taxonomy of the genus level.

As shown by the arrows in Fig. 5, natural *M. aeruginosa* shows various cell shapes and developmental stages of cell division or invagination. The majority of these cells observed in our electron microscopic findings were "two cells about-to-become-four", as do rod-shaped bacteria. In general cell division is symmetrical, and is activated by the septum forming a median constriction, or invagination of the cell membrane. Also, cell wall layers LI and LII participate equally in the pattern of *Gleocapsa alpicola* and *A. nidulans* (Allen, 1968). Although the amounts of wall LI and LII were not quantitatively measured, in fact, layer LII is much greater than that of LI in terms of relative distribution.

Blue-green algae was the first major group of phototrophs to arise with a two-stage photosynthetic pathway capable of oxdizing water to produce molecular oxygen (Giovannoni *et al.*, 1988). The photosynthetic lamellae or thylakoids in the cytoplasm of *M. aeruginosa*, unlike those of other chlorophyllous plants, are not enclosed in membrane-bounded groups to form chloroplasts, nor are they grouped or associated with each other.

Table 2. Comparison of filamentous projections in the outer wall.

	Lounatmaa *et al.* (1980)	Leak (1967)	Golecki (1977)	present study
length	1 μm	0.5 μm	0.15 μm	0.136 μm
diameter	0.6 μm	0.06~0.08 μm	0.06~0.075 μm	0.056 μm
strains	*Synechocytis*	*Anabaena*	*Anacystis*	*Microcystis*

They are widely distributed, are more restricted to the periphery, and sometimes, the bundles of gas vacuoles are divided into two parts, across the proximal to the distal portion of the cytoplasm (Fig. 8). Phycobilisomes were multiprotein complexes and were observed by electron microscopy in the Rhodo- and Cyanophyceae, as discrete structures attached to the stroma surfaces of the lamellae (Gray *et al.*, 1973). But our figures demonstrated either solitary or parallel to each other. Especially, their parallel arrangement between phycobilisomes and lamellae was essentially not face-to-face in the same attached line, but lied between clusters of phycobilisomes and lamellae. Also, they were difficult to distinguish from other subunits dispersed in the nucleoplasm such as ribosomes, electron dense granules or particles. From our observations, it is clear that phycobilisomes are present in *M. aeruginosa*, as accessory pigments, while in some they are absent (Gantt and Conti, 1969), but they are difficult to find in free living samples (Lefort, 1965).

The gas vacuole of *M. aeruginosa* is one of the most well-documented cell organelles, assuming their ability to regulate algal buoyancy according to physical conditions, light intensity, temperature, pH and pressure (Weathers *et al.*, 1977). They were visible as reddish granules in many planktonic blue-green algae and possibly

aided in shading the photosynthetic pigments (Porter and Jost, 1976). The vacuoles in our findings were cylindrical or hexagonal with a round termination. They were mainly distributed in the distal portion, as clusters, or bundles, in the cytoplasm, but sometimes were located proximally (Fig. 5). They ranged from 0.3 to 0.5 μm in length and were about 0.1 μm in diameter (Fig. 6 and 8), similar to those documented by Jone and Jost (1970). The fine structure of other granules or inclusions such as cyanophycin granules, phosphate granules, glycogen granules, ribosomes, lipid deposits and poly-β-hydroxybutyrate granules in *M. aeruginosa* were not seen to a significant degree. However, the ultrastructure of cyanophycin granules and phosphate granules were observed indistinctly after fixation with OsO_4. Cyanophycin granules have an irregular spheroidal form up to about 0.6 to 1.5 μm in diameter, similar to *Anabaena variabilis* in vegetative cells (Leak, 1967). Phos-phate granules also have a more irregularly structured shape, approximately 0.5 μm in diameter. These electron-dense bodies are seen as large and slight staining structures, or metachromatic granules (Fig. 6), similar to the bacterial findings of Jensen (1968). But our results, as regards the concentration of total phosphate, shows hardly any morphological differences between the two samples. After being cultured in the absence of phosphate, few filmentous alga have these granules, whereas, after the same period in the presence of phosphate, such granules are abundant (Talpasayi, 1963). Without much replication, our results indicate that it is difficult to find morphological differences in these granules between natural and culture conditions. In general, a large number of small and dense particles were distributed in the nucleoplasm or centroplasm and near thylakoid lamellae of *M. aeruginosa*. Among them, ribosomes and glycogen granules were seen little, in fact, glycogen granules were more easily stained than the ribosomal particles (Fig. 8 and 9). Glycogen granules were distributed mainly along with thylakoid membranes in the distal portion of cytoplasm (Fig. 9), while both granules were mixed in the proximal region. Although it is difficult to evaluate the exact locale of these granules, more likely the ribosomal particles are distributed near the nucleoplasm than the glycogen granules, and close to thylakoid lamellae. In conclusion, there were no remarkable morphological differences in *M. aeruginosa* sampled from the two natural habitats. *M. aeruginosa* have a number of inclusions, similar to those of cyanobacterial cells and Gram-negative bacteria, although their morphological characteristics have some difference. We conclude that the characteristics of the cell wall outer layer, polyhedral bodies and gas vacuoles may be a useful keys for blue-green algal taxonomy above genus level.

ABSTRACT

In order to understand the morphological differences between two different organic loadings by its upstream, and to compare with other algal groups with references, the fine structure of blue-green algae, *Microcystis aeruginosa* Kützing, taken from two branches, Tongbok and Bosung stream of Lake Chuam, Korea pennisula was examined. It showed extinct differences in most physicochemical factors between both branches, except water temperature and pH values. The concentrations of total phosphorus in Tongbok branch were twice as those of Bosung. *M. aeruginosa* cells were enumerated totally 1.2×10^4 cells/ml and these individuals in branch of

Tongbok were close to two times as much as Bosung. In light and electron microscopy, natural *M. aeruginosa* colonies formed irregular shape and non-directional array in amorphous matrix. They were consisted of many kinds of cells, youngs or olds in cell division, solitary, and various size of cells. Each cell ranged from 2.61 to 5.40 μm in diameter, and averaged as 3.54± 0.19 μm. In cytoplasm, they contained a number of inclusions in various size, shape and appearances. Among them, polyhedral bodies or carboxysomes, a structured granules, photosynthetic lamellae or thylakoids, and gas vacuoles were prominent and easy to recognize. Although it was failed to find the definable morphological variations in the ultrastructure of *M. aeruginosa* in terms of algal habitual environments, some useful characters were founded, outer layer of cell wall, polyhedral bodies and gas vacuoles, in blue-green algal classification and taxonomy.

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FIGURE LEGENDS

Fig. 1. Light micrograph of blue-green algae, *Microcystis aeruginosa* Kützing fixed with FAA. The colonial cells showing irregular form surrounded with amorphous matrix. ×600.

Fig. 2. *M. aeruginosa* colonies has many kinds of granules and bundles of gas vacuoles, widely distributed in cytoplasm. It accidently shows unknown granules whether uptake from cell outside or discharge from cytoplasm (arrow). ×4,000. Scale bar is 1 μm.

Fig. 3. There shows the unknown granules hang on the outer layer of cell wall (arrow), although we don't know whether cytoplasmic granule such as polyphosphate granule, phosphate-accumulating bacteria and sewage bacteria or fault in electron microscope. ×10,000. Scale bar is 1 μm.

Fig. 4. Ultrastructure of ovoidal *M. aeruginosa*. It show the bundle of gas vacuoles resticted to periphery. They contained a number of inclusions in various size, shape and appearances. ×20,000. Scale bar is 1 μm.

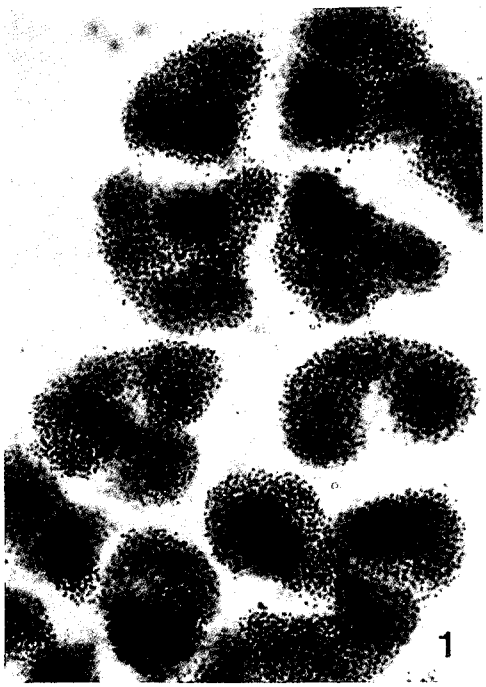
Fig. 5. There shows cytoplasmic invagination or meristemic contraction in cell division of *M. aeruginosa* Kützing. It show a large cyanophycin granule (CG). The bundle of gas vacuole were concentrately distribution in centroplasm (thick arrow), compare to resticed to periphery in Fig. 6. ×15,000. Scale bar is 1 μm.

Fig. 6. Spheroial type of *M. aeruginosa*. It show more resticted to periphery in cytoplasm than Fig. 4. But there is no morphological differences between both cases. ×20,000. Scale bar is 1 μm.

Fig. 7. Cellular coverings are divided into four layers, plasmalemma or cell membranes (CM), peptidoglycan layer (c), and cell wall (b), and filamentous projections similar to pili (a). It show a definable interface (thick arrow) that may seem to be a phenomeon appears in early stage of cell division, and also, gas vacuoles (GV) are prominent peripherally. ×88,000. Scale bar is 0.1 μm.

Fig. 8. They show a number of various dense granules, polyhedral bodies, cyanophycin granules, phycobilisomes or glycogen granules in neuroplasm, even though inconspicuous to the thylakoid lamellae (T) and gas vacuoles (GV). ×40,000. Scale bar is 0.2 μm.

Fig. 9. It shows hexagonal polyhedral bodies (PB), dense cyanophycin granules (CG), gas vacuoles (GV), and a number of small structured granules in nucleoplasm of *M. aeruginosa*. ×60,000. Scale bar is 0.2 μm.



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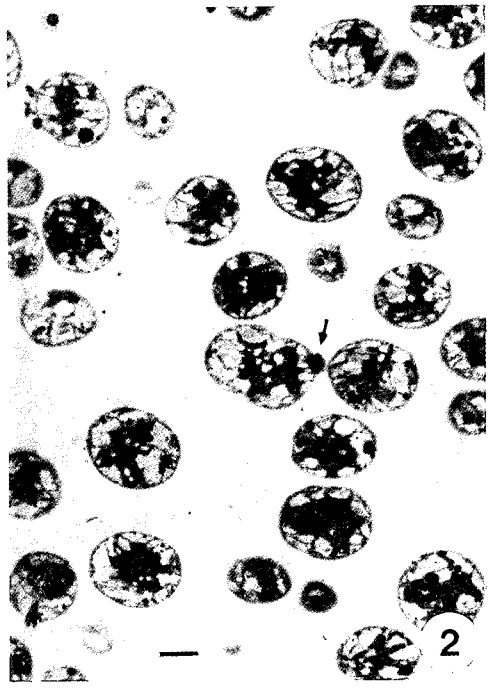

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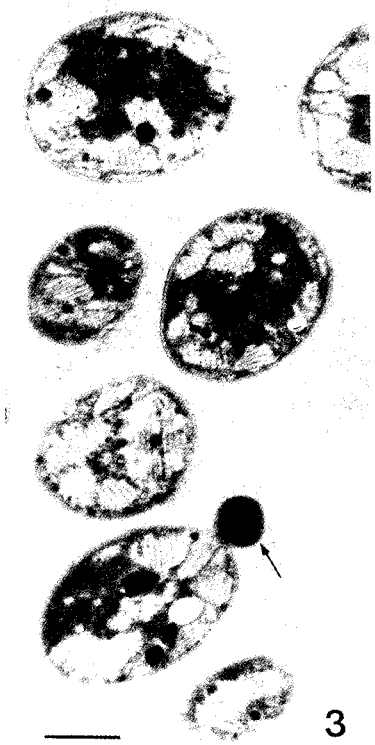

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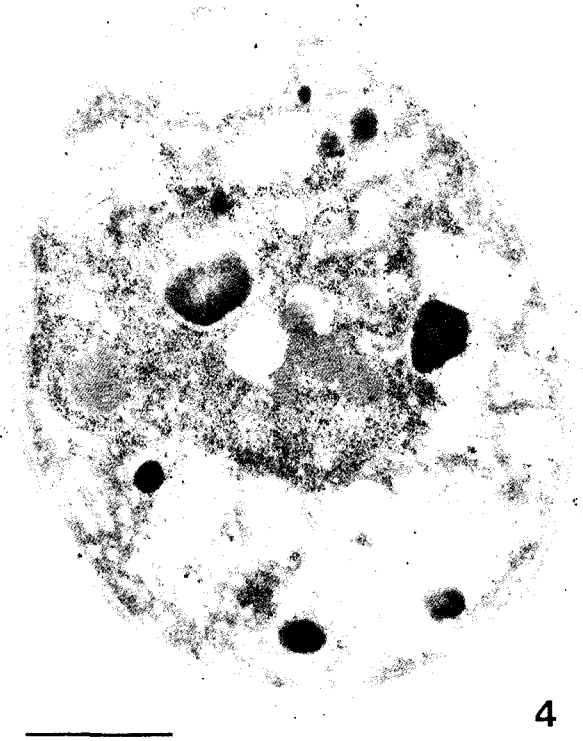

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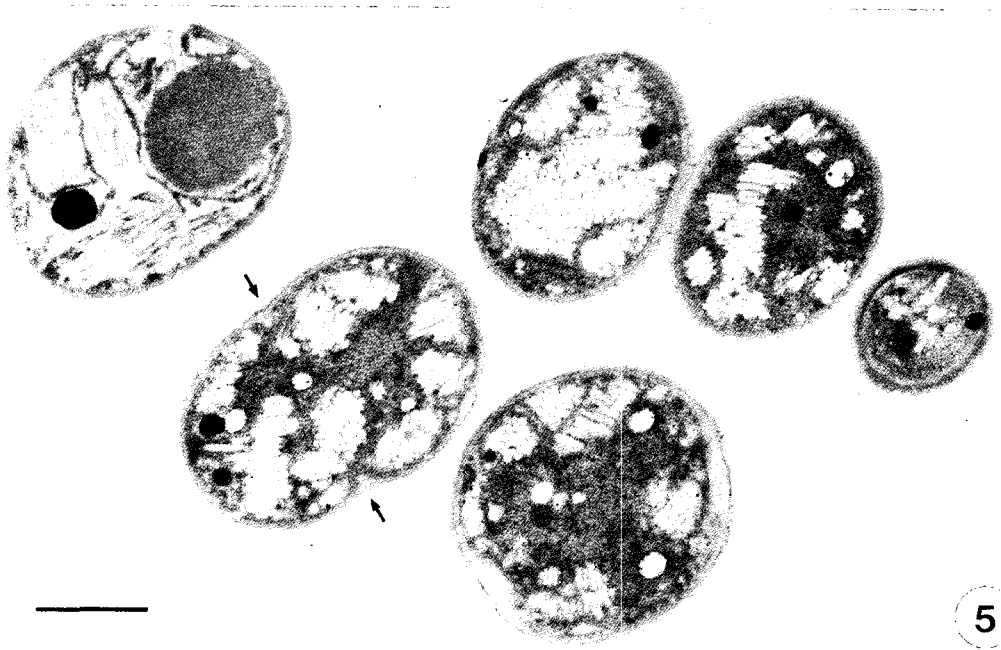

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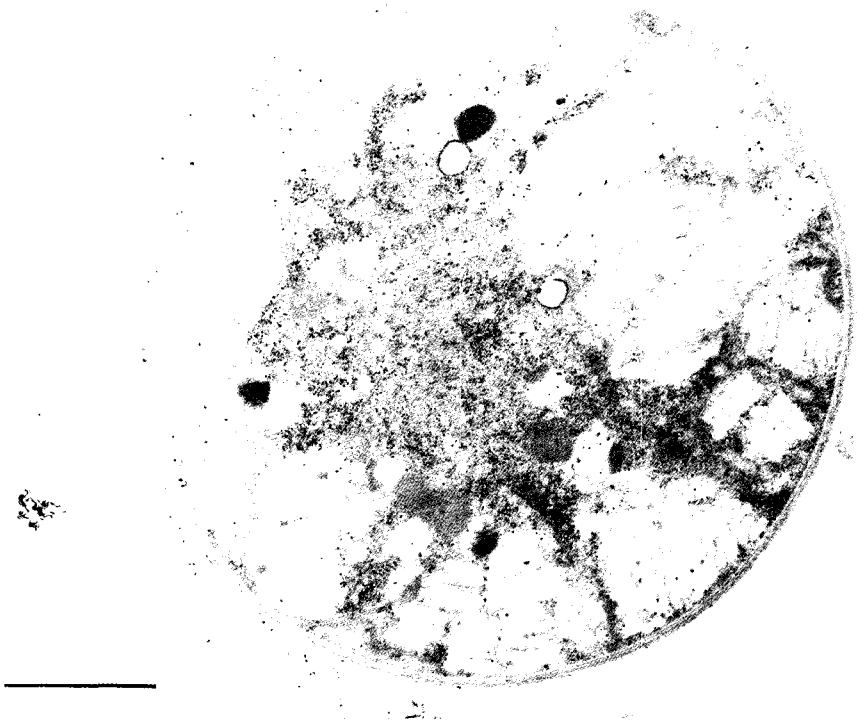

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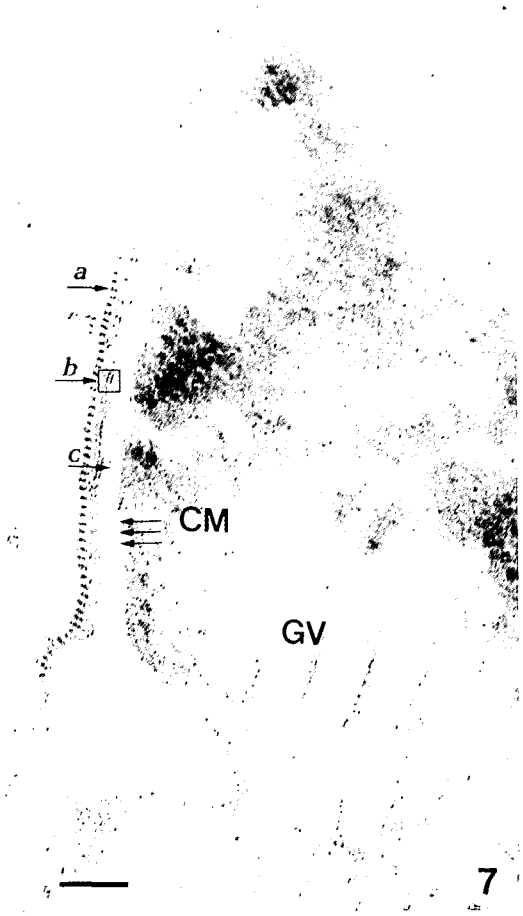

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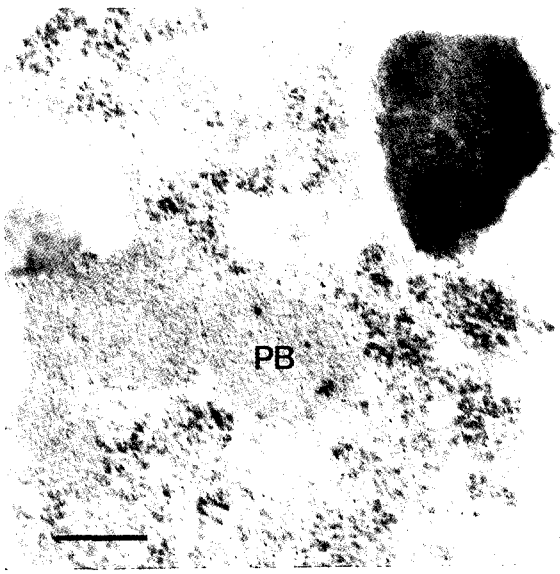

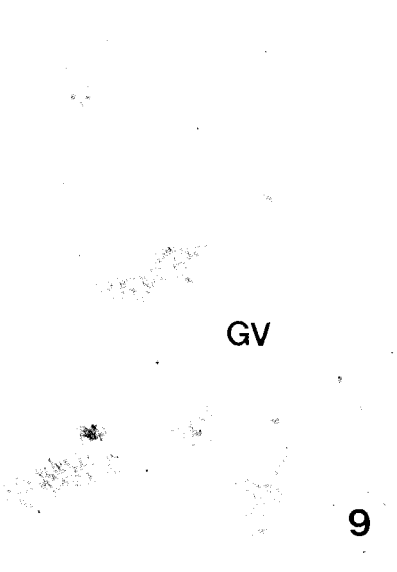

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